Aerobic and Anaerobic Metabolism of *Listeria monocytogenes* in Defined Glucose Medium

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A defined medium with glucose as the carbon source was used to quantitatively determine the metabolic end products produced by *Listeria monocytogenes* under aerobic and anaerobic conditions. Of 10 strains tested, all produced acetoin under aerobic conditions but not anaerobic conditions. Percent carbon recoveries of end products, typified by strain F5069, were as follows: lactate, 28%; acetate, 23%; and acetoin, 26% for aerobic growth and lactate, 79%; acetate, 2%; formate, 5.4%; ethanol, 7.8%; and carbon dioxide, 2.3% for anaerobic growth. No attempt to determine carbon dioxide under aerobic growth conditions was made. The possibility of using acetoin production to assay for growth of *L. monocytogenes* under defined conditions should be considered.

Previous studies (3, 5, 9, 10) of the metabolic end products of growth of *Listeria monocytogenes* have been done for the purpose of identifying unique metabolites and to obtain information to better understand the physiology and classification of this human pathogen found in certain foods (7). Aerobic growth of *L. monocytogenes* in a semidefined medium resulted in production of 10 acids, as well as acetylmethylcarbinol (acetoin) and small amounts of other compounds (3). However, we are unaware of any reports that quantify metabolic end products produced by *L. monocytogenes* in a defined medium under aerobic and anaerobic conditions. *L. monocytogenes* is classified as giving a positive result in the Voges-Proskauer test for acetoin production, a key biochemical test for identification (12). Interpretation of the Voges-Proskauer test can vary according to the quality of reagents but more importantly according to growth conditions, such as whether the inoculum is aerobically grown, the type of medium, and oxygen availability during growth (1, 12, 14). This study was done to define the constitutive metabolism of *L. monocytogenes* by analyzing metabolites produced from glucose in a defined medium under aerobic and anaerobic conditions and to identify key metabolites that indicate *L. monocytogenes* metabolic activity.

The glucose defined medium (GDM) was adapted from the medium described by McFeeters and Chen (8) and modified by the addition of 4.8 μM DL-thioctic acid, which is required for growth by *L. monocytogenes* (11). Medium constituents included ammonium, magnesium, manganese, and iron salts (solution 1); vitamins and cofactors (solution 2); nucleotides (solution 3); amino acids (solution 4); and pH-adjusted phosphate buffer (solution 5). The ingredients were stored frozen as five separate stock solutions at 100× concentrations. Stock solutions were thawed, diluted to the final volume with added glucose carbon source (1%), and filter sterilized through a 0.2-μm-pore-size filter (Costar Corp., Cambridge, Mass.). Other carbon sources substituted for glucose were *N*-acetylglucosamine (1%) and *N*-acetylmuramic acid (1%). All medium constituents were purchased from Sigma Chemical Co. (St. Louis, Mo.). Voges-Proskauer reagents were α-naphthol

(10 mg/ml of 95% ethanol; Fisher Chemical Co., Fairlawn, N.J.), L-arginine (10 mg/ml; Sigma), and KOH (40%; Sigma).

The following 10 strains of *L. monocytogenes* (with their respective serotypes, if known) were used for fermentation balance studies: F5069 (serotype 4b), F5027 (serotype 1a), 675-3 (serotype 1a), DA-1 (serotype 4), and Scott A (serotype 4b) (from C. Donnelly, University of Vermont); UAL 500 and UAL 501 (from M. Stiles, University of Alberta); NCK 157 (from T. Klaenhammer, North Carolina State University); NCF-U2K3 (from P. Foegeding, North Carolina State University); and ATCC 19115 (serotype 4b).

Quantitation of GDM end products after fermentation was done by high-performance liquid chromatography (HPLC) (9). Identification of end-product compounds was done by comparing them to known standards analyzed by three different types of detectors (refractive index, UV absorbance, and conductivity). Carbon dioxide was quantitated according to the method of Fleming et al. (4). Salt concentration was determined by the Fajans adsorption method (2).

Overnight cultures grown in GDM either aerobically or anaerobically at 25°C were used as a 1% inoculum in fresh medium. Samples were taken periodically for HPLC analysis and plate counts. Final samples after 72 h of incubation were used to calculate the fermentation balances. Aerobic incubation was done on a Gyrotory shaker (model G10; New Brunswick Scientific, Edison, N.J.) set at 180 rpm with 20-ml cultures in 50-ml Erlenmeyer flasks (Pyrex) loosely covered with tin foil. Anaerobic incubation was done in sealed tubes (16 by 100 mm; Vacutainer; Becton Dickinson Co., Rutherford, N.J.) deoxygenated and inoculated in an anaerobic hood (Coy Laboratory Products Inc., Grass Lake, Mich.). Colony counts were done by using Oxford medium (Difco, Detroit, Mich.) spread plates with serial dilutions in 0.1% peptone (Difco) diluent. Plates were incubated at 30°C for 48 h.

The data presented in Fig. 1 and Table 1 are for strain F5069 and were typical of all strains tested with regard to types and amounts of end products formed (a range of carbon recoveries is given). *L. monocytogenes* reached a higher maximum population and sustained viability longer if grown aerobically rather than anaerobically (Fig. 1). The maximum cell density of anaerobic cultures was 1 log unit less than the maximum cell density of aerobic cultures, but the specific growth rates were

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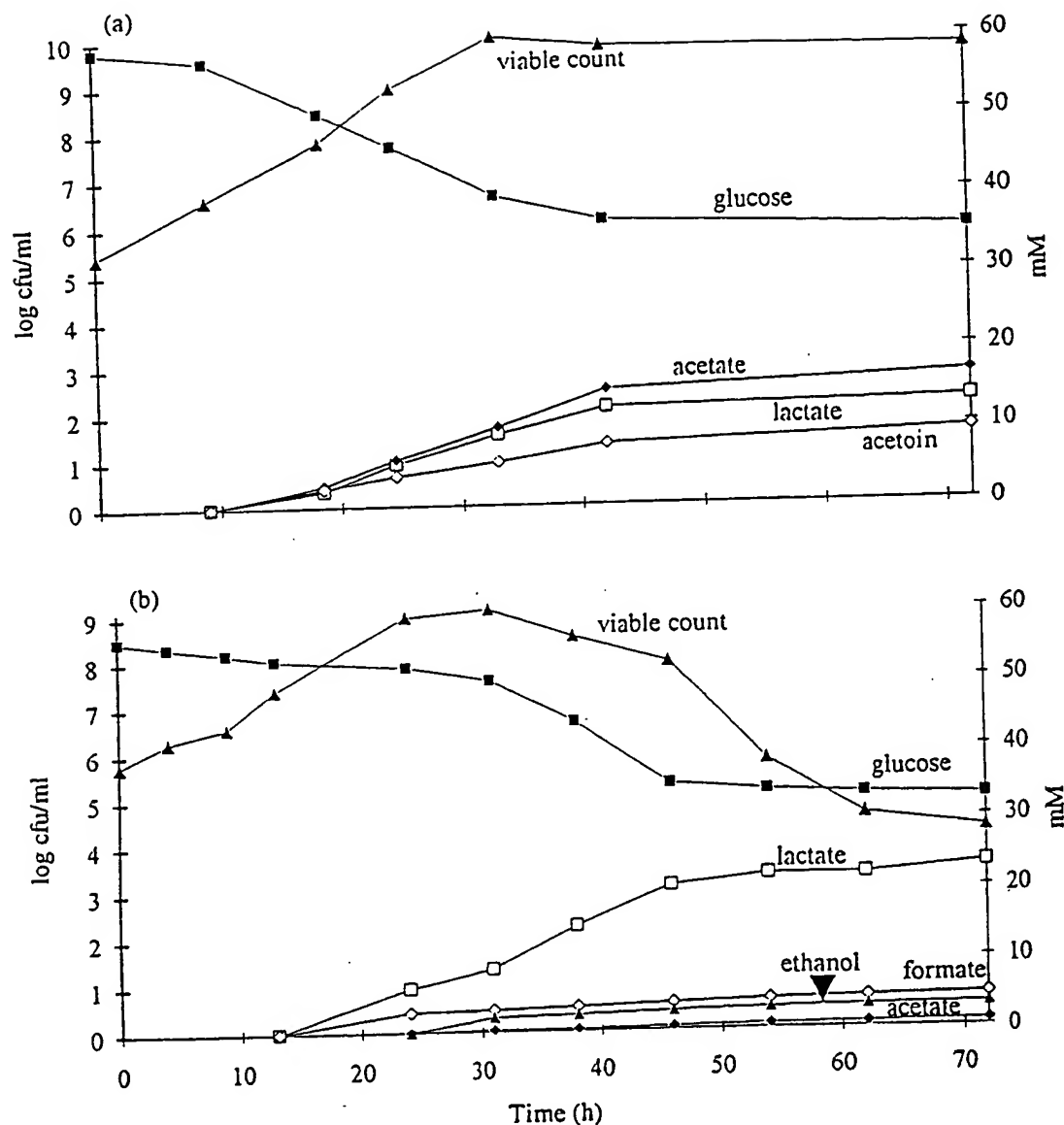† Present address: Hunt-Wesson, Inc., 1645 W. Valencia Dr., Fullerton, CA 92633.

FIG. 1. Aerobic (a) and anaerobic (b) growth and metabolism of *L. monocytogenes* F5069 in GDM.

similar, being 0.33 and 0.27 h^{-1} for aerobic and anaerobic growth, respectively. Specific growth rates in brain heart infusion broth (Difco), a rich medium, were 0.85 and 0.6 h^{-1} for aerobic and anaerobic growth, respectively (data not shown). Although specific growth rates were lower in GDM, they were adequate for completion of the growth phase within 72 h. At that time, a final pH of 4.2 was measured and metabolites were stable (Fig. 1).

Acetoin, rather than 2,3-butanediol, accumulated in the aerobic cultures (Table 1), apparently because of the abundance of oxygen present during the fermentation (1, 12, 13). Acetoin is the reactive compound for the Voges-Proskauer test, which is a key biochemical test for the identification of *L. monocytogenes*. Acetoin is a constitutive metabolite and assayable in GDM as an indicator of aerobic growth of *L. monocytogenes*. An increased yield of lactate relative to yields of other metabolites demonstrated the apparent homofermentative nature of *L. monocytogenes* under anaerobic conditions (5), but other

products represented 17% of the glucose carbon metabolized, indicating that *L. monocytogenes* is not strictly homofermentative. The directly measured carbon recovery for the anaerobic culture was more complete because carbon dioxide could be accounted for in the closed system. Although carbon dioxide was not measured in the aerobic shake flask culture, recovery was near 100% if the assumption was made that 1 mol of CO_2 was produced for each C_2 compound produced. The estimated amount of carbon dioxide generated aerobically was calculated from the stoichiometry of the 2,3-butanediol metabolic pathway, where 2 molecules of pyruvate are needed to form 1 molecule of acetoin with the release of 2 molecules of carbon dioxide (6). Additionally, 1 molecule of carbon dioxide is formed per 1 molecule of acetate. This gives a carbon recovery rate of 102%, which accounts for all glucose carbon metabolized after corrections were made for evaporation losses during fermentation. The concentration of metabolites in the open aerobic culture was adjusted for evaporation by measuring the

TABLE 1. Carbon recoveries from glucose utilization by *L. monocytogenes* F5069 grown aerobically and anaerobically[a]

Parameter[b]	Aerobic			Anaerobic		
	Actual concn (mM)[c]	Total carbon atoms (mM)	% Recovery	Actual concn (mM)	Total carbon atoms (mM)	% Recovery
Glucose used	23.6	141.7		14.8	88.6	
Lactate formed	13.3	39.9	28	23.4	70.1	79
Acetate formed	16.6	33.2	23	0.9	1.8	2.0
Acetoin formed	9.4	37.4	26	0.0	0.0	0.0
Formate formed	0.0	0.0	0.0	4.8	4.8	5.4
Ethanol formed	0.0	0.0	0.0	3.4	6.9	7.8
Carbon dioxide formed	35.3[d]	35.3[d]	25[d]	2.0	2.0	2.3
Carbon recovery, total (range for 10 strains)			77.0 [102[d]] (68.8–77.0)			96.5 (90.6–96.5)

[a] Final pH values were 4.22 for aerobic growth and 4.24 for anaerobic growth. O/R balances, calculated as described by Wood (15), were 0.94 for aerobic growth and 1.29 for anaerobic growth.

[b] An initial glucose concentration of 55.5 mM (1%) was used.

[c] HPLC analyses were carried out after incubation of *L. monocytogenes* in GDM for 72 h at 25°C.

[d] Estimated from aerobic growth based on stoichiometry of 2.3-butanediol metabolic pathway. CO_2 was measured directly when cells were grown anaerobically.

increase in NaCl concentration. The NaCl was added at a low concentration (0.1%) so as not to affect the growth rate significantly and was not metabolized by the culture. Loss due to evaporation averaged 17% over the 72-h incubation time. The ranges of carbon recoveries among the 10 strains tested were 68.8 to 77.0% when cultures were grown aerobically (values not corrected for carbon dioxide production) and 90.6 to 96.5% when cultures were grown anaerobically. The data in Table 1 are for the strain with the highest carbon recovery rates.

The oxidation/reduction (O/R) balance of fermentation end products under aerobic conditions was 0.94 when CO_2 production was assumed as noted in Table 1. However, the O/R balance was 1.29 under anaerobic conditions, which suggests that a reduced product(s) is missing from our analyses. Perhaps hydrogen gas, which we made no attempt to measure, was formed. Formate hydrogen lyase is known to be present in certain bacteria which metabolize glucose via the 2,3-butanediol pathway (6). It also is possible that an analytical error in the HPLC method, particularly in ethanol analysis, could account for the missing reduced product. The classical method for determining O/R values (15) imposes all of the analytical error on the products that are more oxidized or reduced than the substrate fermented. In our experiments, these represented a minor portion of the products formed, while lactic acid accounted for 79% of the carbon recovered (Table 1). Further study is needed to determine if our O/R imbalance is due to some missing reduced compound or to analytical error.

Although various metabolic end products of *L. monocytogenes* growth have been reported (3, 5, 9), this study provides the first known accounting of products produced from glucose in a defined medium under aerobic and anaerobic conditions. Small concentrations of isoacids and isohydroxy acids have been reported, but the compounds may have been amino acid oxidation products of the rich medium used (3). No unique fermentation products were detected from either aerobic or anaerobic utilization of glucose in this study of *L. monocytogenes* growth in a GDM. The data presented in Table 1 represent the constitutive metabolism of *L. monocytogenes* grown in GDM, so that secondary reactions by the organism or within the medium itself are avoided. If other secondary end products were formed, they were not detectable by the HPLC method used.

The ability of *L. monocytogenes* to use a wide variety of carbon sources has been reported (10, 11). When *N*-acetylglu-cosamine and *N*-acetylmuramic acid were substituted for glucose as the sole carbon source, growth was apparent under aerobic and anaerobic conditions after 72 h, with acetoin produced only aerobically. Thus, the defined medium supports growth of the organism on substrates other than glucose. Acetoin can be reliably produced under these defined conditions and is assayable by using Voges-Proskauer reagents directly in the medium. Maximum acetoin production was observed when glucose was at a concentration of 0.6%. Alterations in glucose concentration were not studied anaerobically. Aerobic production of acetoin serves as a key indicator of *L. monocytogenes* metabolic activity when the organism is grown in pure culture aerobically in GDM. The ability to quickly and simply prepare a defined medium from frozen stock solutions which supports the growth of *L. monocytogenes* provides a useful tool for further research on this organism.

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